

# Paeoniflorin ameliorates acquisition impairment of a simple operant discrimination performance caused by unilateral nucleus basalis magnocellularis lesion in rats

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## Abstract

The effect of paeoniflorin on learning impairment of operant brightness discrimination performance was investigated in rats with unilateral nucleus basalis magnocellularis (NBM) lesion. The animals with unilateral NBM lesion exhibited a significant acquisition impairment of brightness discrimination task during the early phase of a training period (1–5 days after starting the training session). When administered daily during a training period, paeoniflorin significantly improved the learning impairment of unilateral NBM-lesioned rats at 0.01 but not 0.1 mg/kg/day (p.o.). Tacrine (0.3 mg/kg/day, p.o.), a cholinesterase inhibitor, also significantly ameliorated the learning deficit. These results suggest that paeoniflorin improves the impairment of non-spatial learning performance caused by cholinergic dysfunction in rats and that it may have a beneficial effect on senile dementia.

**Key words** paeoniflorin, unilateral nucleus basalis magnocellularis lesion, brightness discrimination task, rat.

## Introduction

Neuronal cell loss in the nucleus basalis has been found in the brains of patients suffering from Alzheimer's disease and senile dementia.<sup>1,2)</sup> The nucleus basalis magnocellularis (NBM) sends cholinergic projections to the neocortex in a rat.<sup>3)</sup> In experimental animals, lesion of NBM produces the learning and memory deficits.<sup>4)</sup> For example, NBM lesions cause impairment of working memory,<sup>5,6)</sup> memory retention<sup>7,8)</sup> and selective attention.<sup>9,10)</sup> In addition, a previous report from this laboratory showed that unilateral NBM lesion is sufficient to produce a substantial spatial learning deficit in rats.<sup>12)</sup> These profiles of NBM lesion provide a useful animal model of dementia of the Alzheimer-type.

Paeoniflorin is a major component of the peony root, an herb that has long been used in traditional Chinese prescriptions to treat certain types of dementia. Recent reports from this laboratory demonstrated that perorally administered aqueous extract of peony root and paeoniflorin attenuated scopolamine-induced impairment of radial maze performance in rats<sup>11)</sup> and unilateral NBM lesion-induced impairment of learning performance in the 4-arm baited radial maze task in rats.<sup>12)</sup> These findings suggest that paeoniflorin has a beneficial effect on cholinergic dysfunction-induced memory impairment in spatial cognitive tasks such as a radial maze task. However, no information is available on the effect of paeoniflorin on learning performance impaired by dysfunction of central cholinergic systems in non-spatial cognitive tasks such as an operant discrimination, although

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Ohta *et al.*<sup>13)</sup> reported that paeoniflorin administered p.o. improved acquisition performance of aged rats in an operant brightness discrimination task. In the present study, to further elucidate the effect of paeoniflorin on cholinergic dysfunction-induced impairment of learning performance, we investigated the effect of paeoniflorin on NBM lesion-induced deficit in the discrimination learning in rats, and compared it to that of 9-amino-1,2,3,4-tetrahydroacridine (tacrine), a potent cholinesterase inhibitor.

### Materials and Methods

**Animals** : Male Wistar rats (Japan SLC, Shizuoka) weighing 290–390 g were used. Four rats were housed in a cage with free access to water in an air-conditioned room. Housing conditions were thermostatically maintained at  $23 \pm 1^\circ\text{C}$  with 60 % humidity, in a 12h light/dark cycle (lights on: 07:30–19:30 h). Rats were maintained on a restricted feeding schedule designed to keep their body weight at about 85 % of the free-feeding level. All trials were performed between 09:00–18:00 h. Prior to these experiments, animals were handled for 5–10 min per day for 3 consecutive days.

**Lesioning of NBM** : NBM lesion was performed by slightly modifying the methods described in a previous report.<sup>12)</sup> Briefly, the animals were anesthetized with pentobarbital-Na (35 mg/kg, i.p., Abbott Laboratories, IL, USA) and fixed on a stereotaxic apparatus. Ibotenic acid (7.5  $\mu\text{g}/0.5 \mu\text{l}$ ; Sigma Chem., MO, USA) dissolved in phosphate-buffered saline (pH 7.4) was injected into the unilateral NBM (1.3 mm posterior to the bregma; 2.4 mm lateral to the suture; 7.4 mm ventral to the surface of the cortex) according to the atlas of Paxinos and Watson<sup>14)</sup> over 3 min. The injection needle was left in the place for 6 min after administration. The rats injected with the same volume of phosphate-buffered saline alone were used as the sham-operated group. After completing the behavioral study, coronal brain sections were prepared from the NBM lesioned rats and the location and extent of the lesions were confirmed.

**Apparatus** : As previously reported the rats were trained in a standard rodent Skinner cage (31 $\times$ 29 $\times$ 23 cm, MATYS, Toyama, Japan) equipped with two

retractable levers placed 15 cm apart and 5 cm above the grid floor. In this study, only the right lever was used. Pressing the lever was reinforced by a food pellet (45 mg, Bio-Serve, NJ, USA). A food pellet receptacle was mounted 3 cm above the floor at an equal distance between the levers. The test cage was placed in a sound-proof cubicle equipped with a 6 W house light that was used as a cue signal. A dim light located above the lever was always turned on during each experimental session. Stimulus application, food supply, and recording of lever pressing responses were controlled using a microcomputer.

**Procedures** : The behavioral procedure was detailed elsewhere.<sup>13,15,16)</sup> Briefly, the animals were first adapted to the test cage and trained to press the lever for food reinforcement (FR1). This schedule was progressively increased in ratio until an FR10 schedule was attained. When the number of responses stabilized (more than 20 pressings/min), the rats received either unilateral NBM lesion or sham-operation. After a 1-week recovery period, the pretest session was performed. In this session, the house light was alternately turned on and off in a 3-min intervals for a 24-min session period to assess neophobic reactions to lights off. After the pretest session, brightness discrimination training was started. Each training session consisted of FR10 reinforcement for lever presses during periods when the house light was on (S+) and of no reinforcement for presses during periods when the light was off (S-). The duration of a S+ or S- period was 3 min and each period was presented alternately. The total duration of a session was 24 min. The percentage of incorrect responses, the number of responses during S- periods divided by the number of responses in a session, was calculated.

**Drug tests** : Either paeoniflorin or water was administered perorally (p.o.) 90 min before daily training for acquisition of brightness discrimination performance. When testing tacrine (Sigma Chem., St. Louis, MO, USA), either saline or tacrine was administered p.o. 60 min before daily training. Paeoniflorin and tacrine were dissolved in water ( $\approx$  pH 7.0) just before the start of the experiments, and administered in a constant volume of 0.2 ml/100 g body weight, respectively.

**Statistics** : Differences between the percentages of

incorrect responses were analyzed using two-way analysis of variance (ANOVA).

## Results

### Discrimination learning in NBM-lesioned rats

In the pretest session, the number of responses during the S- period was about 50 % of the responses in NBM-lesioned and sham-operated groups, suggesting that these animal groups exhibit no neophobic reactions to the discriminative stimulus (darkness) (Fig. 1). No significant difference in the final level of the percentage of incorrect responses was observed between NBM-lesioned and sham-operated groups after a 10-day training period. NBM-lesioned rats, however, showed significantly impaired learning performance compared with sham-operated animals in the early phase of training sessions (during a 5-day training period). A two-way ANOVA revealed a

significant group difference in terms of the percentage of incorrect responses [ $F(1,55) = 26.44, P < 0.001$ ] but not in terms of the responses [ $F(1,55) = 1.84, P > 0.05$ ] throughout the early phase of training sessions (from day 1 to day 5).

### Effects of paeoniflorin on discrimination learning impaired by NBM lesion

As shown in Fig. 1, the daily administration of paeoniflorin (0.01 mg/kg, p.o.) attenuated impairment of the discrimination learning observed in NBM-lesioned rats during the early phase of training sessions (from day 1 to day 5). A two-way ANOVA revealed a significant group difference between NBM-lesioned rats with and without paeoniflorin treatment [ $F(1,70) = 17.7, P < 0.001$ ]. This treatment did not affect the total number of responses in NBM-lesioned rats. Paeoniflorin, at a higher dose of 0.1 mg/kg/day, did not produce a significant effect on the impairment of discrimination learning caused by unilateral NBM

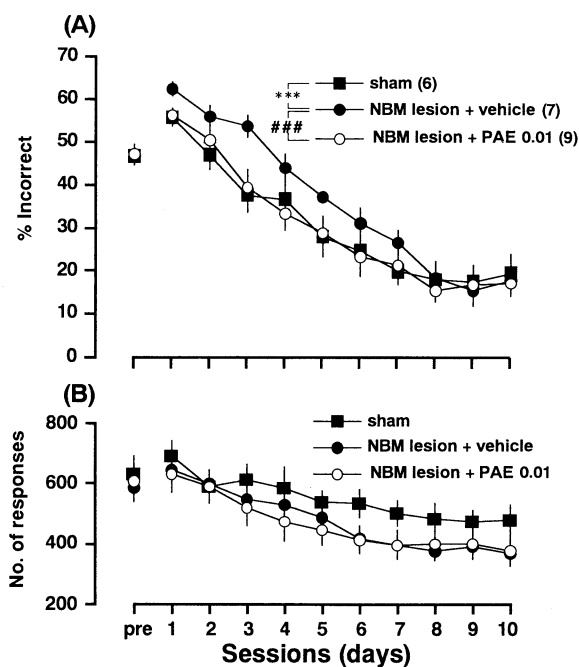


Fig. 1 Effect of paeoniflorin (0.01 mg/kg/day) on brightness discrimination learning in rats with unilateral NBM lesion. Paeoniflorin (PAE: 0.01 mg/kg) was administered perorally 90 min before daily training sessions. The number of animals used are shown in parenthesis. Each data points represents the mean  $\pm$  S.E.M. \*\*\* $P < 0.001$  compared with sham-operated group. ### $P < 0.001$  compared with vehicle-treated NBM lesion group. The % incorrect data obtained in the early phase (from day 1 to day 5) were statistically analyzed.

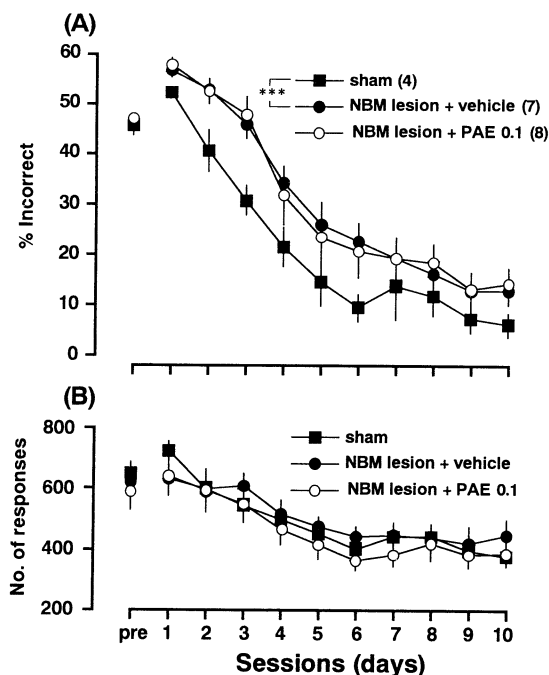


Fig. 2 Effect of paeoniflorin (0.1 mg/kg/day) on brightness discrimination learning in rats with unilateral NBM lesion. Paeoniflorin (PAE: 0.1 mg/kg) was administered perorally 90 min before daily training sessions. The number of animals used are shown in parenthesis. Each data points represents the mean  $\pm$  S.E.M. The % incorrect data obtained in the early phase (from day 1 to day 5) were statistically analyzed. \*\*\* $P < 0.001$  compared with sham-operated group.

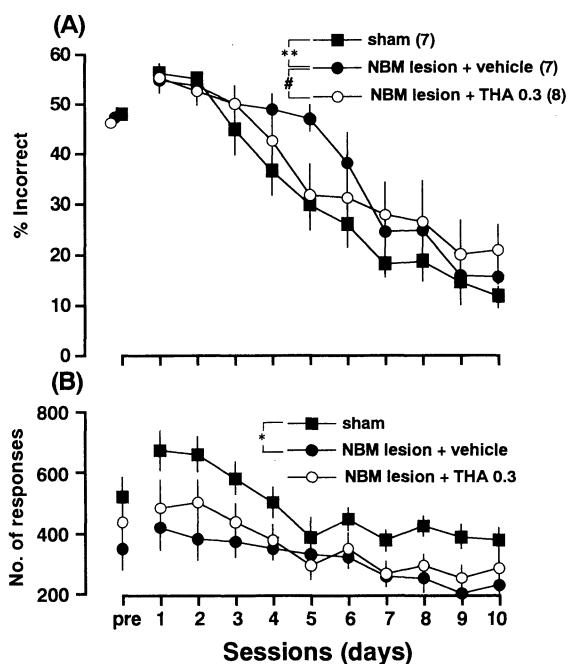


Fig. 3 Effect of tacrine on brightness discrimination learning in rats with unilateral NBM lesion. Tacrine (THA : 0.3 mg/kg) was administered perorally 60 min before daily training sessions. The number of animals used are shown in parenthesis. Each data point represents the mean  $\pm$  S.E.M. The data obtained in the early phase (from day 1 to day 5) were statistically analyzed. \*\* $P < 0.01$  and \* $P < 0.05$  compared with sham-operated group. # $P < 0.05$  compared with vehicle-treated NBM lesion group.

lesion [ $F(1,59) = 0.012$ ,  $P > 0.05$ ; Fig. 2]. No significant difference in the number of responses was observed among groups.

#### Effects of tacrine on discrimination learning impaired by NBM lesion

The daily administration of 0.3 mg/kg (p.o.) tacrine, a reference drug, significantly improved impairment of discrimination learning observed in NBM-lesioned rats during the early phase of training sessions (from day 1 to day 5). A significant group difference in the percentage of incorrect responses was observed between NBM-lesioned rats with and without tacrine treatment [ $F(1,65) = 4.04$ ,  $P < 0.05$ , Fig. 3].

### Discussion

NBM provides primary cholinergic projections to the neocortex in rats<sup>3)</sup> and lesions of this nucleus are

known to cause deficits in learning and memory performance in a variety of behavioral tasks.<sup>7,12,17,18)</sup>

This NBM lesion model has widely been utilized in an attempt to develop new cognitive enhancing drugs effective for the treatment of the Alzheimer disease. In the present study, the daily administration of paeoniflorin (0.01 mg/kg, p.o.), as well as daily administration of the cholinesterase inhibitor tacrine, significantly improved impairment of operant discrimination performance caused by unilateral NBM lesion in rats. These findings further support the idea that paeoniflorin may be useful for treatment of dementia caused by cholinergic dysfunction.

In this study, unilateral NBM lesion caused impairment of acquisition of a simple operant discrimination performance in rats and this impairment was observed only in the early phase of training sessions. This finding agrees with the data reported by Santucci and Haroutunian<sup>19)</sup> and Butt and Hodge.<sup>18)</sup> The latter authors showed that bilateral ibotenic acid lesions of NBM impaired acquisition performance in an operant discrimination task in rats and that lesioned rats were performing as well as sham groups by the last test day. Thus, it is conceivable that unilateral lesion of NBM produces a qualitatively similar deficit in acquisition performance in a non-spatial cognitive task to bilateral NBM lesions. Ohta *et al.* demonstrated using a 4-arm baited radial maze task that a spatial learning unilateral NBM lesion caused deficits in rats and that this deficit was due to impairment of both working and reference memories. Moreover, they found that paeoniflorin ameliorated the unilateral NBM lesion-induced deficits in working and reference memories.<sup>12)</sup> Taken together, the present results suggest that paeoniflorin produces beneficial effects on acquisition impairments of both spatial and non-spatial cognition caused by NBM lesion in rats.

The daily peroral administration of tacrine (0.3 mg/kg) significantly improved acquisition impairment in adult rats with unilateral NBM lesion, indicating a role of cholinergic systems in acquisition performance of rats in an operant discrimination task. This result is in contrast to the previous findings in this laboratory<sup>13,15)</sup> that scopolamine did not impair discrimination learning in young rats and that daily

administration of tacrine (0.3 and 1 mg/kg, i.p.) failed to improve impairment of discrimination learning in aged rats in which dysfunction of central cholinergic systems is well accepted.<sup>20,21)</sup> An exact reason for this discrepancy remains unclear but it may be due to involvement of not only cholinergic systems but also other neuronal systems in aging- and scopolamine-induced deficits in learning and memory.<sup>22,23)</sup>

Daily treatment with 0.1 mg/kg paeoniflorin had no significant effect on unilateral NBM lesion-induced acquisition impairment in an operant discrimination task in this study. This result is consistent with the data that daily administration of paeoniflorin at the same dose did not improve acquisition performance of aged rats in the same task.<sup>13)</sup> In a previous study, we observed a bell-shaped dose response relationship in the action of paeoniflorin on scopolamine-induced spatial cognitive impairment.<sup>11)</sup> Thus, it will be interesting to test the effect of paeoniflorin using doses lower than 0.01 mg/kg.

In conclusion, the present results indicate that paeoniflorin improves the impairment of non-spatial learning performance caused by unilateral NBM lesion in rats. These findings suggest the therapeutic potential of paeoniflorin in the treatment of Alzheimer-type disease. Nevertheless, further investigation will be required to clarify the mechanism underlying the ameliorative action of paeoniflorin on unilateral NBM lesion-induced non-spatial cognitive impairment.

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### 和文抄録

片側前脳基底核(NBM)破壊ラットにおける明暗弁別課題習得障害に対する芍薬成分ペオニフロリンの効果を検討した。NBM破壊ラット群では弁別課題習得訓練開始の初期(訓練開始5日後まで)において有意な習得障害が認められた。訓練開始から1日1回訓練開始の90分前にペオニフロリン0.01 mg/kgをNBM破壊群に経口投与することにより習得障害が有意に改善されたが0.1 mg/kgの用量では有意な効果は認められなかった。一

方、参照薬として用いたコリンエステラーゼ阻害薬タクリン(0.3 mg/kg/day, p.o.)を投与したNBM破壊群においても習得障害が有意に改善された。これらの成績はペオニフロリンが中枢コリン神経系の障害によって発現する非空間的な学習課題の習得障害に対しても有効であり、老年痴呆の治療において有用である可能性を示唆する。

### References

- 1) Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T., DeLong, M. R.: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Annal. Neurol.* **10**, 122-126, 1981.
- 2) Whitehouse, P.J., et al.: Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**, 1237-1239, 1982.
- 3) Fibiger, H.C.: The organization and some projections of cholinergic neurons of the mammalian forebrain. *Brain Res. Rev.* **4**, 327-388, 1982.
- 4) Murray, C.L., Fibiger, H.C.: Learning and memory after lesions of the nucleus basalis magnocellularis: reversal by physostigmine. *Neuroscience* **14**, 1025-1032, 1985.
- 5) Beninger, R.J., Jhamandas, K., Boegman, R.J., El-Defrawy, S.R.: Effects of scopolamine and unilateral lesions of the basal forebrain on T-maze spatial discrimination and alternation in rats. *Pharmacol. Biochem. Behav.* **24**, 1353-1360, 1986.
- 6) Biggan, S.L., Beninger, R.J., Cockhill, J., Jhamandas, K., Boegman, R.J.: Quisqualate lesions of rat NBM: selective effects on working memory in a double Y-maze. *Brain Res. Bull.* **26**, 613-616, 1991.
- 7) Miyamoto, M., Kato, J., Narumi, S., Nagaoka, A.: Characteristics of memory impairment following lesioning of the basal forebrain and medial septal nucleus in rats. *Brain Res.* **419**, 19-31, 1987.
- 8) Roberts, A.C., et al.: The effects of excitotoxic lesions of the basal forebrain on the acquisition, retention and serial reversal of visual discriminations in marmosets. *Neuroscience* **34**, 311-329, 1990.
- 9) Robbins, T.W., et al.: Comparative effects of ibotenic acid- and quisqualic acid-induced lesions of the substantia innominata on attentional function in the rat: further implications for the role of the cholinergic neurons of the nucleus basalis in cognitive processes. *Behav. Brain Res.* **35**, 221-240, 1989.
- 10) Pang, K., Olton, D.S., Egeth, H.: Attention and the nucleus basalis magnocellularis. *Society for Neuroscience Abstracts.* **17**, 137, 1991.
- 11) Ohta, H., Ni, J.-W., Matsumoto, K., Watanabe, H., Shimizu, M.: Peony and its major constituent, paeoniflorin, improve radial maze performance impaired by scopolamine in rats. *Pharmacol. Biochem. Behav.* **45**, 719-723, 1993.
- 12) Ohta, H., Nishi, K., Matsumoto, K., Shimizu, M., Watanabe, H.: Paeoniflorin improves learning deficit in 4-arm baited radial maze performance in rats with unilateral nucleus basalis magnocellularis lesion. *Phytomedicine* **1**, 117-121, 1994.
- 13) Ohta, H., Matsumoto, K., Shimizu, M., Watanabe, H.: Paeoniflorin attenuates learning impairment of aged rats in operant brightness discrimination task. *Pharmacol. Biochem. Behav.* **49**, 213-217, 1994.

- 14) Paxinos, G., Watson, C.: "The rat brain in stereotaxic coordinates", Academic Press, San Diego, 1986.
- 15) Ohta, H., Matsumoto, K., Watanabe, H.: Impairment of acquisition but not retention of a simple operant discrimination performance in aged Fischer 344 rats. *Physiol. Behav.* **54**, 443-448, 1993.
- 16) Nitta, H., Matsumoto, K., Shimizu, M., Ni, X.-H., Watanabe, H.: *Panax ginseng* extract improves performance of aged Fischer 344 rats in radial maze task but not in operant brightness discrimination task. *Biol. Pharm. Bull.* **18**, 1286-1288, 1995.
- 17) Hepler, D.J., Wenk, G.L., Gribbs, B.L., Olton, D.S., Coyle, J.T.: Memory impairments following basal forebrain lesions. *Brain Res.* **346**, 8-14, 1985.
- 18) Butt, A.E., Hodge, G.K.: Acquisition, retention, and extinction of operant discriminations in rats with nucleus basalis magnocellularis lesions. *Behav. Neurosci.* **109**, 699-713, 1995.
- 19) Santucci, A.C., Haroutunian, V.: Nucleus basalis lesions impair memory in rats trained on nonspatial and spatial discrimination tasks. *Physiol. Behav.* **45**, 1025-1031, 1989.
- 20) Michalek, H., Fortuna, S., Pintor, A.: Age-related differences in brain choline acetyltransferases, cholinesterase and muscarinic receptor sites in two strains of rats. *Neurobiol. Aging* **10**, 143-148, 1989.
- 21) Stephens, D.N., Weidmann, R., Quartermain, D., Sarter, M.: Reversal learning in senescent rats. *Behav. Brain Res.* **17**, 193-202, 1985.
- 22) Li, H.-B., Matsumoto, K., Tohda, M., Yamamoto, M., Watanabe, H.: NMDA- but not AMPA-receptor antagonists augment scopolamine-induced spatial cognitive deficit of rats in a radial maze task. *Brain Res.* **725**, 268-271, 1996.
- 23) Marcyniuk, B., Mann, D.M.A., Yates, P.O.: Loss of nerve cells from locus coeruleus in Alzheimer's disease in topographically arranged. *Neurosci. Lett.* **64**, 247-252, 1986.